PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
10292.204-WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK 03/00519	01.08.2003	01.08.2002
International Patent Classification (IPC) or both national classification and IPC		
C12Q1/68		
Applicant		
NOVOZYMES A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

EPO - DG 1

15. 10. 2004

(37)

3. This report contains indications relating to the following items:

   I ☒ Basis of the opinion
   II ☐ Priority
   III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☐ Lack of unity of invention
   V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   VI ☐ Certain documents cited
   VII ☐ Certain defects in the international application
   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24.02.2004	14.09.2004
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK 03/00519

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-25 as originally filed

Sequence listings part of the description, Pages

1-2 as originally filed

Claims, Numbers

1-44 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 37-44

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 37-44

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-36
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-36
Industrial applicability (IA)	Yes:	Claims	1-36
	No:	Claims	-

2. Citations and explanations

see separate sheet

Re Item I

Basis of the opinion

The basis of this International Preliminary Examination Report is the application as originally filed.
The present application relates to a method for isolating a polynucleotide that encodes a polypeptide of interest which comprises a signal sequence for secretion or partial secretion.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 37-44 were not searched (see International Search Report). Said claims are therefore not further examined (Art. 34(4)(i)(ii), Art. 17(2)(a) and Rule 66.1(e) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement.

Reference is made to the following documents:

D1: WO 01 77315 A (NOVOZYMES AS), published 18 October 2001.

D2: Frank et al., 'Rapid amplification of plasmid and phage DNA using Phi29 DNA polymerase and multiply-primed rolling circle amplification', GENOME RESEARCH, vol. 11, no. 6, June 2001, pp. 1095-1099.

D3: WO 00 15779 A (UNIV YALE), published 23 March 2000.

D4: US-A-5 648 245 (FIRE ANDREW ET AL), published 15 July 1997.

D5: Zhidong et al., 'Amplification of closed circular DNA in vitro', NUCLEIC ACIDS RESEARCH, vol. 26, no. 4, 15 February 1998, pp. 1126-1127. An abstract of said document has been cited in the International Search Report.

V.1. Independent claim 1 and claims 2-36 which are directly or indirectly dependent thereon appear new in the sense of Article 33(2) PCT as none of the available prior art discloses the features of claims 1-36.

V.2. Document D1 (the reference in parentheses applying to this document) which is considered as the closest prior art relevant to the method of independent claim 1 discloses a method for identifying and isolating a gene of interest from a gene library, wherein the gene encodes a polypeptide carrying a signal sequence for secretion or partial secretion, the method comprising the steps of:

a) providing a genomic DNA library or a cDNA library,

b) inserting into said library a DNA fragment comprising a promoterless and secretion signal-less polynucleotide encoding a secretion reporter,

c) introducing the library comprising the inserted DNA fragment into a host cell,

d) screening for and selecting a host cell that secretes or partially secretes the active secretion reporter,

e) identifying the gene of interest into which the secretion reporter was inserted in the selected host cell, by sequencing the DNA flanking the inserted DNA fragment, and

f) isolating the complete gene of interest identified in step e) (claim 1). The method can be performed using any gene library known in the art (page 16, line 13), preferably, the DNA or cDNA library is comprised in a plasmid (e.g. examples 5 and 8).

The method of present claim 1 differs from that of document D1 only in that the genomic DNA/cDNA library is amplified by rolling circle before or after the DNA fragment comprising a promoterless and secretion signal-less polynucleotide is inserted into said library.

It is to be noted that the fact that in step (a) of the method of claim 1 the library is produced *in vitro* without ultraviolet irradiation of the component polynucleotide, is not seen as a limiting feature of claim 1 as said step of producing the library is not considered as being part of the method of claim 1, in view of the claim's wording.

The application indicates that the effect of such an amplification step by rolling circle is to circumvent the need for intermediate amplification host cells (page 2, lines 7-11). However, such an intermediate amplification host cells is not required by the method of document D1 either. Therefore, the problem to be solved may only be seen as the

provision of an alternative method for identifying and isolating a gene of interest from a gene library, wherein the gene encodes a polypeptide carrying a signal sequence for secretion or partial secretion.

It is well known in the art that gene libraries can be amplified by rolling-circle (e.g. document D3 which discloses a method for amplifying libraries of cloned nucleic acids based on rolling circle (page 14, line 32-page 15, line 19 and page 29, line 7-page 30, line 21); document D5 which discloses a method of amplifying closed circular DNA in vitro, in particular cDNA libraries (abstract and page 1127, col. 2) or document D2 which discloses rolling circle amplification of plasmid and phage DNA (abstract)) and that amplification by rolling circle results in the formation of concatamers (e.g. document D2 (page 1095, col. 1, 2nd paragraph) or document D4 which discloses a method for constructing an oligonucleotide concatamer library by rolling circle (col.1, lines 13-20). It appears therefore that amplifying the DNA/cDNA library by rolling circle is merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed, in particular as the advantage thus achieved can be readily contemplated in advance (i.e. in vitro amplification (e.g. by rolling circle) does not require the use of an intermediate host cell).

The subject-matter of claim 1 does therefore not involve an inventive step (Article 33(3) PCT).

V.3. The features of dependent claims 2-7, 9-14, 21-28 and 30-36 are already known from document D1 (claims 3-29).
Claims 2-7, 9-14, 21-28 and 30-36 are therefore not considered inventive in the sense of Article 33(3) PCT.

V.4. Furthermore, the additional features of dependent claim 8 are implicit features of the vector used to construct the DNA or cDNA library.
Therefore, dependent claim 8 is also considered as not involving an inventive step in the sense of Article 33(3) PCT.

V.5. The additional features of dependent claims 15-20 fall within the scope of customary practice followed in the art.

Claims 15-20 are thus not considered inventive in the sense of Article 33(3) PCT.

V.6. Further comments

A lack of clarity in the meaning of Article 6 PCT arises in claim 18 due to a back reference to claim 18.